Original Research Article

The leaf Essential Oils and Antibacterial Activity of *Juniperus oxycedrus* ssp. *macrocarpa* (S. et Sm.) BalI. Growing in Oum El Bouaghi (semi- arid area), Algeria

ABSTRACT

Leaf essential oils of *Juniperus oxycedrus* (Cupressaceae) wild grown in the region of Oum El Bouaghi (semi- arid area) in Algeria have been analysed by GC-MS. Fifty seven compounds were identified in the leave oils. The leaf oils were mainly composed of 5-Tetradecen-1-ol, acetate, (Z)- (12.9%) ç-Murolene (9.1%), α-Cadinol (5.1%) (ñ)-Cadinene (3.9 %) and some other compounds which were only present in minor amounts. The antimicrobial activity of the essential oils were evaluated by the disc diffusion method and tested against Gram-negative *Escherichia coli, Pseudomonas aerogenosa* and Gram-positive *Staphylococcus aureus* bacteria. Results showed that *Staphylococcus aureus* was the highly resistant to the essential oil.

Aims: This study aimed to extracted, identification of essential oils of Leaves of J. oxycedrus L. ssp. macrocarpa (S. et Sm.) BalI. growing in Oum El Bouaghi (semi- arid area), Algeria and evaluation of their antibacterial capacity.

Results: The GC/MS analysis of the Leaves of J. oxycedrus L. (yielded 0, 36 %) permitted the identification of fifty seven components. The composition and percentage of the compounds are listed by their order of retention times. The main constituents of the essential oil were composed of 5-Tetradecen-1-ol, acetate, (Z) - (12.9%) ç-Murolene (9.1%), α-Cadinol (5.1%) (ñ)-Cadinene (3.9 %) and some other compounds were only present in minor amounts.

Conclusion: The results of analysis of the components Leaves of *Juniperus oxycedrus* ssp. *macrocarpa* (S. et Sm.) BalI. growing in Oum El Bouaghi (semi- arid area), Algeria, permitted the identification of fifty seven components. The essential oil showed that *Staphylococcus aureus* was highly resistant.

Keywords: Juniperus oxycedrus ssp. macrocarpa L., *Essential oil, Chemical composition, GC/MS, Antibacterial activity, Oum El bouaghi.*

1. INTRODUCTION

Essential oils are aromatic oily liquids, volatile, characterized by a strong odour, rarely coloured, and generally less dense than water. They can be synthesized by all plant organs (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots) and therefore extracted from these parts, where they are stored in secretory cells, cavities, canals, epidermic cells orglandular trichomes [1]. Essential oils have a complex composition, containing from a dozen to several hundred components. The great majority of components identified in essential oils includes terpenes (oxygenated or not), with monoterpenes and sesquiterpenes prevailing. Nevertheless, allyl- and propenylphenols (phenylpropanoids) are also important components of some essential oils [2]. The well-known families rich in essential oil are Apiaceae, Asteraceae, Hypericaceae, Lamiaceae, Lauraceae, Myrtaceae, Pinaceae, Piperaceae, Rutaceae, Santalaceae, Zingiberaceae, Zygophyllaceae and Cupressaceae [3, 4]**.**

The genus *Juniperus* (Cupressaceae) consists of approximately 67 species and 28 varieties. The genus is divided into three sections: *Caryocedrus* Edlicher (with only one species); *Juniperus* (syn: *Oxycedrus* Spach with 12 species) and *Sabina* (Miller) Spach (with 55 species) [5]. The genus *Juniperus* (Cupressaceae) is represented in the flora of Algeria by five species, namely *J. Oxycedrus* L.,J*. Sabina* L*., J. thurifera* L*., J. phoenica* L*. and J. communis* L*.,* [6]. *Juniperus oxycedrus* is a shrub or small tree growing wild in stony places of the Mediterranean and Near East countries [7]. In folk medicine *J. oxycedrus* was used for the treatment of various diseases, such as hyperglycaemia, obesity, tuberculosis, bronchitis and pneumonia [8]. Leaves and stems of *J. oxycedrus ssp. macrocarpa* have been found to reduce the blood pressure of normotensive rats, to inhibit the response to hista- mine, serotonin and acetylcholine, and to exhibit significant anti-inflammatory activity [9].

2. MATERIAL AND METHODS

2.1 Plant material

The leaves of *J. oxycedrus ssp. macrocarpa* were collected in May 2018 (fructification stage) in Oum El Bouaghi (longitude: 7°06′48; latitude: 35°52′31; elevation: 925 m; annual precipitation: 412, 66 mm; semi-arid area), Algeria. A voucher specimen was deposited at the life sciences and nature Department, University Larbi Ben M'hidi, Oum el Bouaghi, Algeria under the code number ZA 135 (Figure 1).

Figure 1. Leaves of *Juniperus oxycedrus* ssp. *macrocarpa* (S. et Sm.) Ball.

2.2 Extraction

Essential oils were obtained by hydrodistillation of 100g of dried leaves using a Clevenger-type apparatus for 3 h. The oil was dried over anhydrous sodium sulphate and stored in sealed vials protected from the light at −4 °C before analyses. The oil sample was subsequently analyzed by GC-MS.

2.3 Identification of components

2.3.1 Gas chromatography/mass spectrometry (GC/MS)

The oil was analyzed by GC/MS using an Agilent 5973EI mass selective detector coupled with an Agilent GC6890A gas chromatograph, equipped with a cross-linked 5% PH ME siloxane HP-5MS capillary column (30m x 0.32mm, film's thicknesses 0.25µm). Operating conditions: The carrier gas flow was 1.6 ml He/min, column pressure was 100 Kpa. The injector and detector temperatures were 220°C and 250°C respectively. The column temperature was held at 60°C for 1 min, then raised from 60°C to 200°C at 10°C/min and held there for 5 min and from 200°C to 240°C at 10°C /min and held there for 6 min. The program was run in the splitless mode with a mass range of 50–400 u, and the scan interval was 0.5 s. Detector voltage was set at 1.5 kV (Table 1).

Table 1. General information on GC-MS analysis performed

Column type	**HP-5MS(5% dimethylpolysiloxane) 30m * 0.32mm*0.25 µm**
Injection volume	1µL
Injector temperature	220°C
Detector temperature	250°C
Mode of injection	Split
Vector gaz	Helium

2.3.2 Identification of components

Identification of oil components was achieved on the basis of their retention times Rt, and by comparison of their mass spectral fragmentation patterns with those reported in the literature and stored on the MS library (NIST database). The concentration of the identified compounds was computed from the GC peak total area without any correction factor.

2.4 Antimicrobial activity

2.4.1 Microorganism strains

All of the bacteria; standard strains *E. coli* ATCC 25922, *P. aerugenosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 (Table 2) were obtained from Bacteriology Laboratory Constantine Hospital University (C.H.U). The bacterial strains were first grown on Muller Hinton medium (MHI) at 37°C for 24 h prior to seeding on to the nutrient agar. A sterile 6-mm-diameter filter disk (Whatman paper no. 3) was placed on the infusion agar seeded with bacteria, and each extract suspended in water was dropped on to each paper disk (40 µL per disk) for all of prepared concentrations (8, 4, 2, 1, 0.5, 0.25 µL /mL). The treated Petri dishes were kept at 4°C for 1 h, and incubated at 37°C for 24 h. The antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the disks. Each experiment was carried out in triplicate.

Table 2. Microbial strains used in this experiment

Strain	Reference
E. coli	ATCC 25922
P. aerugenosa	ATCC 27853
S. aureus	ATCC 25923

3. RESULTS AND DISCUSSION

3.1 Identification of components

The GC/MS analysis of the Leaves of J. oxycedrus L. (yielded 0, 36 %) permitted the identification of fifty seven components. The composition and percentage of the compounds are summarized in Table 3. They are listed by their order of retention times. The main constituents of the essential oil were composed of 5-Tetradecen-1-ol, acetate, (Z) - (12.9%) ç-Murolene (9.1%), α-Cadinol (5.1%) (ñ)-Cadinene (3.9 %) and some other compounds were only present in minor amounts (Table 4).

The composition of leaf essential oils of section *Juniperus* is generally much simpler and dominated by simple monoterpenes, in contrast to the essential oils of section Sabina, were oxygenated monoterpenes (e.g. camphor) and sesquiterpenes (e.g. cadinols, cedrol) are the major constituents [9, 10].

According to previous studies on the *Juniperus oxycedrus* species, it has been deduced that the essential oils leaves of this species is very different from one region to another, it is dominated by α-pinene in spain,25-43% [10], in croitia (41.4%) [11], (19.6-55.3) in Portugal [12], (31.25%) in morocco [13], more over in other countries this oil is dominated by lemonene myrcene and manoyl oxide [14] . On the other hand, in the same region we notice that there are a lot of variations between these main compounds; it has been reported that the leaf essential oil of *Juniperus oxycedrus* from Aures (semi arid region)-Algeria- was dominated by manoyl oxide (23.5%), followed by pentadecan-2-enone 6Z (12.6%), abietatriene (8.0%), abieta- 8,11,13-triene-7-one (6.5%), cubebol (4.6%), epi-torilenol (3.8%) and a-cadinol (2.6%)[15]. While other studies found that leaves oil of *J. oxycedrus* growing in El kala (humid area) – Algeria- are characterized by high levels of Germacrene D [16]. Further findings, showed that the main components of essential oil from Djelfa - Algeria, located at the foot of the Saharan Atlas, were respectively trans-pinocarveol (7.0%), cis-verbenol (6.3%) and manoyl oxide (6.0%) [17].

It is renowned that the genotype, organ, season of collection, and geographic position Climatic conditions the applied extraction technique have a considerable effect on the composition [18, 19].

Table 3. Chemical composition of the essential oil of *J. oxycedrus* L. leaves growing in Oum El Bouaghi (semi arid area)

Pic	Chemical constituents	Rt	%
1	Acetone	3.086	1.7
2	α-Pinene	3.788	0.3
3	Cyclopropyl 4-picolyl ketone	4.971	0.2
4	α-Phellandrene	5.253	0.3
5	Benzene, 1-methyl-2-(1-methylethyl)-	5.680	0.8
6	α-Thujene	5.882	0.5
7	D-Limonene	5.945	0.8

8	α-Campholenal	8.815	0.2
9	L-pinocarveol	9.503	0.2
10	2-Methyl-3-(3-methylenebicyclo[3.2.1]oct-6-en-8-yloxy)cyclohex-2-enone	9.864	0.1
11	Acetylfuran	10.654	0.1
12	1,2,4,5-Tetrazin-3-amine, 6-methyl-	11.053	0.1
13	2-Hexanol, 3,3,5-trimethyl-2-(3-methylphenyl)-	11.372	0.1
14	L-α-terpineol	11.863	0.2
15	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	16.817	0.6
16	Neohexane	19.181	0.1
17	L-α-terpineol	20.483	2.8
18	α-Cubebene	22.369	1.3
19	Cyclohexasiloxane, dodecamethyl-	22.472	0.6
20	α-Bourbonene	22.668	2.4
21	1,5-Heptadiene, 2,5-dimethyl-3-methylene-	23.260	0.2
22	α-Maaliene	23.649	0.7
23	Caryophyllene	24.447	1.5
24	1,2,5-Thiadiazolidine, 2,5-di-tert-butyl-, 1,1-dioxide	24.933	0.1
25	Germacrene D	25.077	0.6
26	Copaene	25.868	0.1
27	α-Caryophyllene	26.271	1.8
28	(E)-2-Phenyl-2-butene	27.000	0.2
29	**ç-Muurolene**	27.794	**9.1**
30	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-,	29.166	2.8
31	2,3,4-Trifluorobenzoic acid, cyclobutyl ester	29.880	0.1
32	α-Farnesene	30.137	0.4
33	**(ñ)-Cadinene**	30.414	**3.9**

34	Nonane, 1-iodo-	30.660	0.2
35	3,8-Dimethyl-1,2,3,4-tetrahydro-.gamma.-carboline	30.844	0.2
36	2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol	31.633	3.0
37	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	32.694	1.7
38	Caryophylleneoxide	32.801	2. 5
39	3-Bromomethyl-3,6,6-trimethyl-cyclohexene	33.449	1.5
40	3-Isopropylidene-tricyclo[4.3.1.1(2,5)]undecan-10-one	33.653	0.1
41	Epicedrol	33.782	0.9
42	Bicyclo[2.2.2]octane, 1-methyl-4-(methylsulfonyl)-	34.471	1.6
43	Andrographolide	35.113	3.1
44	Aromadendreneoxide-(2)	37.501	2.0
45	**α-Cadinol**	38.209	**5.1**
46	5-Pentadecen-7-yne, (Z)-	39.317	0.4
47	**5-Tetradecen-1-ol, acetate, (Z)-**	40.207	**12.9**
48	Cyclohexanepropanol-	41.362	0.5
49	2,3-Dihydrofarnesol	41.480	0.8
50	2-Nonadecanone	42.134	0.8
51	3-Ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	43.798	1.8
52	Cyclopentane, (2-methylbutylidene)-	45.517	0.2
53	Diheptyl phthalate	48.476	0.4
54	Lavandulyl acetate	49.325	0.4
55	1H-Naphtho[2,1-b]pyran, 3-ethenyldodecahydro-3,4a,7,7,10a-pentamethyl-	53.070	3.0
56	7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene	55.050	1.0
57	Pimar-15-en-8-yl acetate	56.087	0.3
	Total		80.3 %

Table 4. The main essential oil of the Leaves of *Juniperus oxycedrus* ssp. macrocarpa (S. et Sm.) Ball growing in Oum el Bouaghi

Oil compound	Percentage (%)
5-Tetradecen-1-ol, acetate, (Z)	12.9
ç-Murolene	9.1
α-Cadinol	5.1
(ñ)-Cadinene	3.9
other compound	minor amounts

3.2 Antibacterial activity

Although some volatile compounds have proven to be effective against microorganisms, many studies are still based on the effect of the presence of some phenolic substances and oxygen compounds [1].The diffusion test was applied to three microorganisms including Gram-positive, -negative bacteria. The results are summarized in Table 5 which show that the volatile oil from *Juniperus oxycedrus* prevented the growth of all the tested Gram - microorganisms except *Staphylococcus aureus* (Gram +) and it has been revealed that the obtained inhibition zone varied from 7.00 to 10.00 mm with a highest inhibition zone recorded with *Escherichia coli* at µl/mL.

Although, these results differ from those obtained at the same species growing in North Western Algeria [20, 21]. This may come back to the many differences in the chemical composition of this species.

These results correspond with those obtained on *Juniperus oxycedrus growing in Tunisia in the same condition (*Semi-arid) which show that ***Staphylococcus aureus*** was highly resistant [22].

Table 5. Antibacterial activity of *Juniperus oxycedrus* grown in Oum El Bouaghi

Bacterial strains	**Concentration of JOEB (µl/ml)**					
	8	**4**	**2**	**1**	**0,5**	**0,25**
***Staphylococcus aureus* ATCC 25923**	-	-	-	-	-	-
***Escherichia coli* ATCC 25922**	10±0	10±0	9,67±0,57	7,67±0,57	7,33±0,57	7±0
***Pseudomonas aeruginosa* ATCC 27853**	9±0	8,67±0,57	8,33±0,57	-	-	-

4. CONCLUSION

Our study by GC/MS on *Juniperus oxycedrus* leaves growing in Oum El Bouaghi (Algeria) showed the presence of 57 compounds of the essential oil. The antimicrobial activity of the essential oils was tested against 3 bacteria: *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aerugenosa* through diffusion test disk. The essential oil showed that *Staphylococcus aureus* was highly resistant.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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